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# Physical and functional interaction of the Werner syndrome protein with poly-ADP ribosyl transferase

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**Abstract** **Werner's syndrome is a rare disease of premature ageing. The *WRN* gene product defective in this disorder belongs to the RecQ helicase family and is thought to be involved in DNA metabolism. Another protein, which plays an important role in both DNA replication and repair, is the poly-ADP ribosyl transferase. Here we demonstrate an interaction of these two proteins resulting in ADP-ribosylation of the WRN protein. These results imply that WRN is involved in DNA replication and in DNA repair.**


*Key words:* Werner's syndrome; WRN; Poly-ADP ribosyl transferase; ADP-ribosylation

## 1. Introduction

Werner's syndrome (WS) is a progeroid disorder of the adult, characterized by premature ageing [1,2]. Cells from these patients show chromosomal instability [3–5], and a prolonged S-phase [6].

The gene product, WRN, was shown to have an ATPase activity, a $3'\rightarrow5'$ helicase activity [7] as well as a $3'\rightarrow5'$ exonuclease activity [8,9]. In which kind of cellular processes the protein participates is unknown, recombination, replication and repair are candidates.

The WRN helicase is able to unwind fork structures, RNA/DNA duplexes and alternative DNA structures including tetraplex and triplex DNA, 'bubbles' and Holliday junctions [10–15]. The WRN exonuclease has been reported to digest some of the double stranded intermediate DNA substrates, unwound by the helicase, for example tetraplex and triplex DNA [16].

Several interaction proteins have been reported for WRN. Most of them, such as proliferating cell nuclear antigen (PCNA) [17], DNA polymerase δ [9], replication protein A (RPA) [18], and flap endonuclease 1 (FEN-1) [19], participate in DNA replication and repair. A protein also involved in these two processes is the poly-ADP ribosyl transferase (pADPRT). It is known that this enzyme is activated in the presence of DNA strand breaks. Upon activation pADPRT transfers ADP ribose units from $NAD^+$ onto itself and other proteins, thereby mostly inactivating the target protein [20].

pADPRT was shown to be a component of the multiprotein DNA replication complex (MRC) [21]. This complex among others also contains some proteins interacting with WRN. We therefore anticipated that pADPRT might be a potential interaction protein for WRN. Here we report that WRN indeed physically interacts with pADPRT and becomes ADP-ribosylated in the presence of DNA breaks.

## 2. Materials and methods

### 2.1. Chemicals and antibodies

All chemicals were purchased from Merck, Roth or Sigma unless otherwise indicated.

Polyclonal WRN antibodies against the N-terminal fragment, representing the first 411 amino acids, and pADPRT antibodies against the DNA binding domain were raised in rabbits and purified against the antigens over a cyanobromide-activated Sepharose column.

### 2.2. Cell lines and culture conditions

Cell lines used: Wi38 was purchased from the American Type Culture Collection, KoHe and LW4 [22] were established by M. Hirsch-Kauffmann, Innsbruck, Austria from skin biopsies.

The cells were grown in minimal essential medium (MEM-Earle) with 10% fetal calf serum (Biochrom AG, Germany) and cultivated at 37°C in an atmosphere of 5% $CO_2$ in air. All cells were free of mycoplasms.

### 2.3. Coimmunoprecipitation

Nuclear extract (220 μg), isolated as described [23], was incubated with 5 μg pADPRT antibodies. The precipitate was loaded onto a 6% sodium dodecyl sulfate (SDS)–polyacrylamide gel and was used for a Western blot following electrophoresis. The blot was incubated with rabbit anti-WRN antibodies. Alkaline phosphatase-conjugated anti-rabbit antibodies were used for detection.

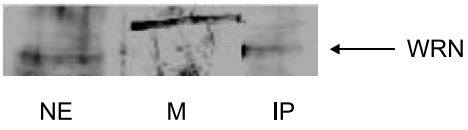


Fig. 1. Immunoprecipitation of WRN with pADPRT antibodies. Nuclear extract (NE) from Wi38 is loaded on the left lane to locate the WRN protein, and the immunoprecipitate (IP) is loaded on the right one. The arrow points to the WRN protein band detected by WRN antibodies. Middle lane: marker band of MW 204 000 (M).

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*Abbreviations:* FEN-1, flap endonuclease 1; MRC, multiprotein DNA replication complex; NHEJ, non-homologous end joining; pADPRT, poly-ADP ribosyl transferase; PAGE, polyacrylamide gel electrophoresis; PCNA, proliferating cell nuclear antigen; RPA, replication protein A; WRN, Werner protein; WS, Werner's syndrome

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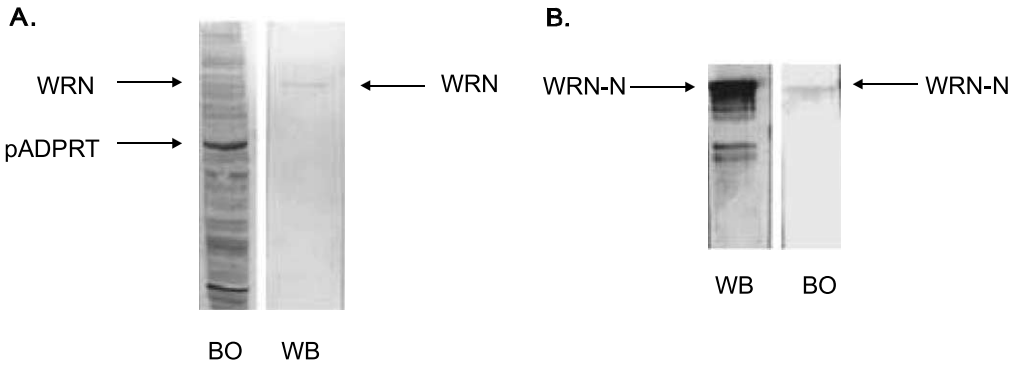


Fig. 2. Blot overlay (BO) experiments to detect an interaction between WRN and pADPRT. A: Nuclear extracts were loaded on a gel and blotted onto a nitrocellulose membrane. Left lane: WRN and other pADPRT-interacting proteins detected by pADPRT antibodies after incubation of the gel with purified pADPRT (BO). Right lane: Western blot (WB). WRN directly detected with WRN antibodies. B: Same procedure as in A using an N-terminal fragment of WRN (WRN-N). Left lane: Western blot (WB) of the purified fragment. Right lane: blot overlay (BO).

### 2.4. Western blot

Nuclear extracts (60 μg protein) were fractionated in 10% SDS–polyacrylamide gel electrophoresis (PAGE) and subsequently blotted onto a nitrocellulose membrane (Schleicher and Schuell). Ponceau S staining was used to confirm uniform protein loading. The blots were probed with anti-WRN and anti-pADPRT antibodies, and immunoreactive complexes were analyzed with alkaline phosphatase-conjugated anti-rabbit antibodies.

### 2.5. Blot overlay

Nuclear extract or the WRN N-terminal fragment was loaded onto a 6% SDS–polyacrylamide gel. After electrophoresis the gel was blotted onto a nitrocellulose membrane. The blot was stained with Ponceau S and the lanes dedicated to be blot overlaid were cut off. Those lanes were blocked in overlay buffer (50 mM Tris–HCl pH 7.5, 0.5% bovine serum albumin, 0.25% gelatin, 0.2% Triton X-100, 2.5 mM β-mercaptoethanol) for 4 h at 4°C. Purified pADPRT in overlay buffer was allowed to incubate overnight at 4°C. The membranes were washed four times with Tris-buffered saline at room temperature before a Western blot was performed using WRN antibodies.

### 2.6. ADP-ribosylation assay

Nuclear extract (50 μg) from Wi38 fibroblasts or 5 μg WRN N-terminal fragment was incubated with 1 μg sonified herring sperm DNA, 1 μCi [$^{32}$P]NAD$^{+}$ and in the case of the WRN fragment with 5 μg recombinant pADPRT at 30°C for 20 min. The probes were used for SDS–PAGE. The wet gel was exposed to a phosphoimager screen, which was developed by a phosphoimager.

## 3. Results

Since it was hypothesized that both WRN and pADPRT are involved in DNA replication and DNA repair we were interested in investigating a possible interaction between these proteins. Actually, we could demonstrate an interaction by means of a coimmunoprecipitation (Fig. 1). In the right lane (immunoprecipitate, IP) the antibodies identified a protein with the size of WRN (MW 160 000). The left lane shows the endogenous position of the WRN protein in nuclear extracts.

This interaction was confirmed by blot overlay. Nuclear extract or the purified N-terminal fragment of WRN was loaded on an SDS–polyacrylamide gel, blotted onto nitrocellulose membranes and stained with Ponceau S. The blots were cut into two parts. One part was directly used for a Western blot, developed with WRN antibodies; the second part was incubated with purified pADPRT before a Western blot was performed and developed with pADPRT antibodies. When comparing the Western blots developed with WRN antibodies and the blot overlay developed with pADPRT antibodies, a protein of the size of WRN could be detected in both blots (Fig. 2A). That this interaction was not restricted to embryonic lung fibroblasts (Wi38) could be proved by the use of

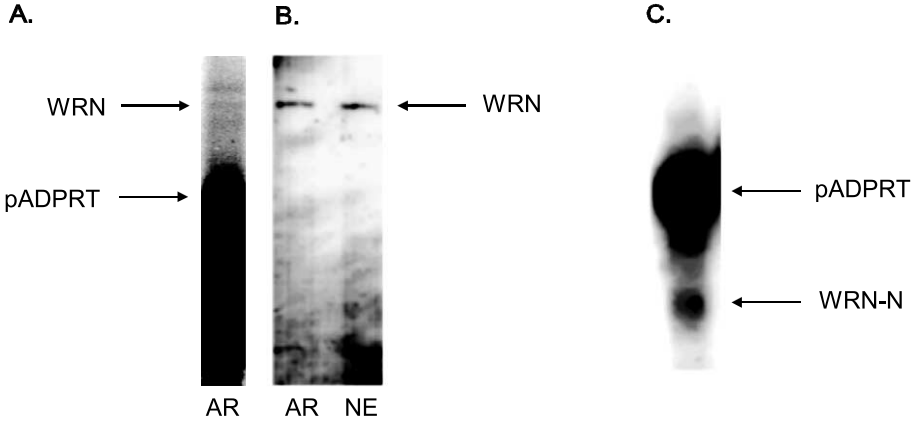


Fig. 3. ADP-ribosylation of WRN. A: Nuclear extract from Wi38 was incubated with [$^{32}$P]NAD$^{+}$ and sonified herring sperm DNA, loaded on a gel, and following electrophoresis exposed to a phosphoimager screen plate (AR). The bands were compared with the WRN bands on the Western blot (B) derived from the same gel. Left lane: ADP-ribosylation probe (AR). Right lane: Nuclear extract (NE). Arrows indicate WRN bands. C: ADP-ribosylation of the N-terminal WRN fragment (WRN-N).

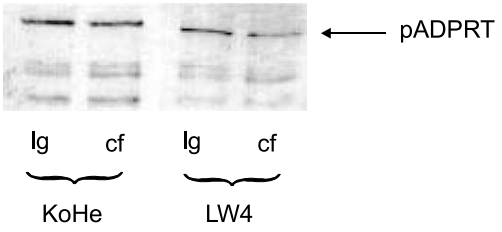


Fig. 4. Western blots for pADPRT in confluent and logarithmically growing control and WS fibroblasts. In the control cell line (KoHe) equal amounts of pADPRT were detected in the logarithmic (lg) and confluent (cf) states. In the WS cell line (LW4) the pADPRT amount in the confluent cells appears to be reduced. Ponceau S staining confirmed uniform protein loading (not shown).

material from other tissues such as primary skin fibroblasts and blood cells (data not shown). The blot overlay of the N-terminal WRN fragment with pADPRT antibodies also resulted in a positive signal (Fig. 2B).

Being aware of an interaction between WRN and pADPRT the question arose whether WRN was modified by pADPRT. Therefore an ADP-ribosylation assay was performed. Nuclear extracts were incubated with [$^{32}$P]NAD$^{+}$ together with sonified herring sperm DNA, in order to activate the endogenous pADPRT. After SDS–PAGE the wet gel was exposed to a phosphoimager screen plate and blotted thereafter. On the phosphoimage (Fig. 3A) a couple of $^{32}$P-labelled bands could be distinguished. To clarify if one of the bands represents WRN, we used the gel to perform a Western blot and developed the proteins with WRN antibodies (Fig. 3B). Comparison of the blot with the phosphoimage revealed one of the radioactively labeled bands to be WRN. An ADP-ribosylation assay was also performed with the purified N-terminal WRN fragment. In this case pADPRT activity was recruited by purified recombinant pADPRT instead of nuclear extracts. As seen in Fig. 3C the WRN fragment was ADP-ribosylated too.

Since WRN and pADPRT apparently interact but WS cells do not have any functioning WRN protein, we investigated if the amount of pADPRT in cells derived from WS patients differed from that of control cells. We isolated nuclear extracts from both confluent and logarithmically growing cells from controls and from authentic WS fibroblasts [22]. In comparison to the control cell line we could detect a reduction of pADPRT in the WS cell line at confluency (Fig. 4).

## 4. Discussion

The nuclear proteins WRN and pADPRT are both related to central cellular processes. pADPRT has been identified for instance as a component of MRC [21] and WRN copurifies with this complex [17]. Here we could show the direct interaction of these two proteins in human embryonic lung fibroblasts (Wi38), a result that was very recently verified by a report by Lebel and coworkers [24] using embryonic kidney cells. Our own results obtained with other tissues point to the importance and universality of this interaction.

pADPRT ADP-ribosylates several components of the MRC, for instance RNA polymerase α, topoisomerase I and PCNA. This modification is purposed both to regulate the activity of the proteins during replication and to regulate the composition of the MRC. Here, to our knowledge for the first time, we document that pADPRT is also able to ADP-ribosylate WRN. Some components of the MRC such as RPA, FEN-1 and DNA polymerase δ have been shown to interact with WRN [17–19,25]. This, together with the fact that WRN has been located to sites of replication in the nucleoli [25–27], points to a role of WRN in replication. ADP-ribosylation of WRN may regulate its participation in this process.

Since we [22,28] and others [29–32] have shown that cells from patients suffering from WS are sensitive to X-ray, alkylating agents, etc., it was proposed that WRN is also involved in repair processes. The most probable repair pathway WRN participates in is the non-homologous end joining (NHEJ). This repair pathway is used in the repair of DNA double strand breaks. Thereby, WRN interacts with Ku70/80 [33,34], a component of the NHEJ, which stimulates the exonuclease activity of WRN [35].

ADP-ribosylation of diverse proteins in the presence of free DNA strand breaks tends to result in modification of their activities in DNA replication or repair [36–39]. The ADP-ribosylation of WRN as a participant in replication and repair therefore may act as a switch signal for WRN to stop its engagement in the replication machinery for the sake of becoming part of the repair process. This, for instance, could be achieved by inactivation of the helicase activity and activation of the exonuclease activity of the WRN protein following ADP-ribosylation.

The interaction between WRN and pADPRT entails a diminution of pADPRT in WRN-deficient fibroblasts. This may explain both the delayed induction of p53 after DNA damage and the attenuation of p53-mediated apoptosis in WS cells [40,41]. Increasing chromosomal instability and cancer-proneness are disastrous consequences.

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